abcam

Product datasheet

Anti-RAB10 antibody [MJF-R23] ab237703

KO VALIDATED Recombinant RabMAb

14 References 11 Images

Overview

Product name	Anti-RAB10 antibody [MJF-R23]
Description	Rabbit monoclonal [MJF-R23] to RAB10
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A549, HeLa, HCT 116, MCF7, NIH/3T3, PC-12 and C6 whole cell lysates. ICC/IF: A549 and MCF7 cells. Flow: A549 cells. IP: A549 whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. This antibody was developed with support from The Michael J. Fox Foundation. THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	MJF-R23
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab237703 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/600.
IP		1/30.
ICC/IF		1/500.
WB		1/1000. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).

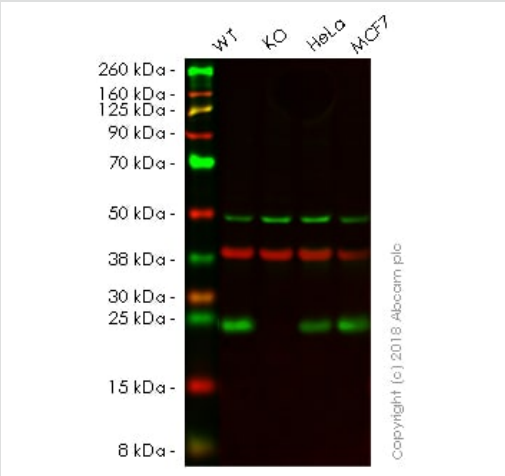
Target

Function The small GTPases Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membranes. Rabs cycle between an inactive GDP-bound form and an active GTP-bound form that is able to recruit to membranes different set of downstream effectors directly responsible for vesicle formation, movement, tethering and fusion (By similarity). That Rab is mainly involved in the biosynthetic transport of proteins from the Golgi to the plasma membrane. Regulates, for instance, SLC2A4/GLUT4 glucose transporter-enriched vesicles delivery to the plasma membrane. In parallel, it regulates the transport of TLR4, a toll-like receptor to the plasma membrane and therefore may be important for innate immune response. Plays also a specific role in asymmetric protein transport to the plasma membrane within the polarized neuron and epithelial cells. In neurons, it is involved in axonogenesis through regulation of vesicular membrane trafficking toward the axonal plasma membrane while in epithelial cells, it regulates transport from the Golgi to the basolateral membrane. Moreover, may play a role in the basolateral recycling pathway and in phagosome maturation. According to PubMed:23263280, may play a role in endoplasmic reticulum dynamics and morphology controlling tubulation along microtubules and tubules fusion.

Sequence similarities Belongs to the small GTPase superfamily. Rab family.

Cellular localization Cytoplasmic vesicle membrane. Golgi apparatus membrane. Golgi apparatus, trans-Golgi network membrane. Endosome membrane. Recycling endosome membrane. Cytoplasmic vesicle, phagosome membrane. Cell projection, cilium. Endoplasmic reticulum membrane. Associates with SLC2A4/GLUT4 storage vesicles (PubMed:22908308). Localizes to the base of the cilium (PubMed:20576682). Transiently associates with phagosomes (By similarity). Localizes to the endoplasmic reticulum at domains of new tubule growth (PubMed:23263280).

Images



Western blot - Anti-RAB10 antibody [MJF-R23] (ab237703)

All lanes : Anti-RAB10 antibody [MJF-R23] (ab237703) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : RAB10 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

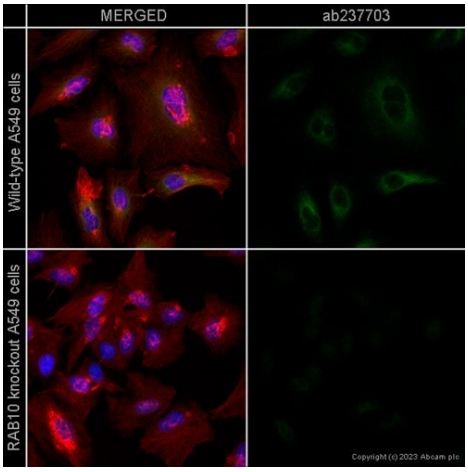
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 22 kDa
Observed band size: 25 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab237703 observed at 25 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

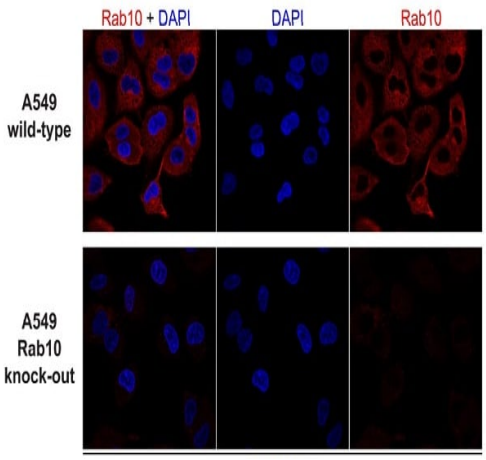
ab237703 was shown to recognize RAB10 in wild-type A549 cells as signal was lost at the expected MW in RAB10 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and RAB10 knockout samples were subjected to SDS-PAGE. Ab237703 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-RAB10 antibody [MJF-R23] (ab237703)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% saponin permeabilized RAB10 KO A549 (RAB10 knockout human lung carcinoma epithelial cell) (**ab261868**) labelling RAB10 (red) with ab237703 at 0.04 µg/ml, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) at 1/1000 dilution. The nuclear counter stain is DAPI (blue).

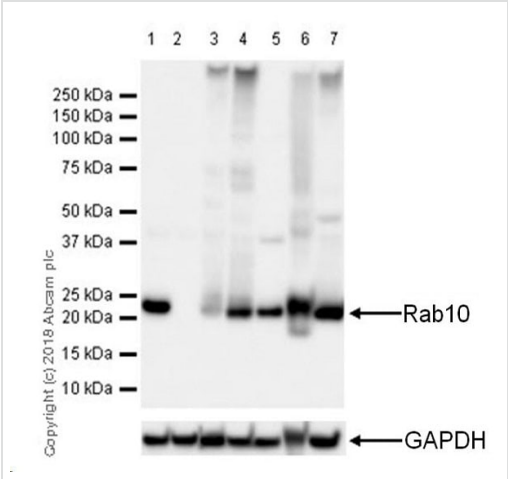
Confocal image showing cytoplasmic staining in wild-type A549 cell line, and no staining in RAB10 KO A549 cell line. Images were acquired on the Perkin Elmer® Operetta HCA and a maximum intensity projection of 7 confocal planes is shown for the representative images.



Immunocytochemistry/ Immunofluorescence - Anti-RAB10 antibody [MJF-R23] (ab237703)

Image courtesy of Dr. Dario Alessi from University of Dundee

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% saponin permeabilized A549 wild-type and knock-out cells labeling RAB10 (red) with ab237703 at 0.5 µg/ml, followed by anti-Rabbit secondary at 1/1000 dilution. The nuclear counter stain is DAPI (blue).



Western blot - Anti-RAB10 antibody [MJF-R23] (ab237703)

All lanes : Anti-RAB10 antibody [MJF-R23] (ab237703) at 1/1000 dilution

Lane 1 : Wild-type A549 (human lung carcinoma epithelial cell line) whole cell lysate
Lane 2 : Rab10 knockout A549 whole cell lysate
Lane 3 : HeLa (Human cervix adenocarcinoma epithelial cell line) whole cell lysate
Lane 4 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate
Lane 5 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
Lane 6 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 7 : C6 (rat glial tumor glial cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 10 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

The WT and Rab10 KO A549 lysates were kindly provided by our collaborator Dr. Dario Alessi, University of Dundee.

Western blot - Anti-RAB10 antibody [MJF-R23] (ab237703)

Anti-RAB10 antibody [MJF-R23] (ab237703) at 1/10000 dilution + HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate at 15 µg

Secondary

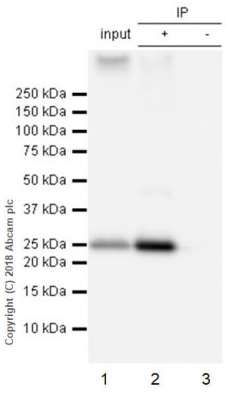
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 22 kDa

Observed band size: 22 kDa

Exposure time: 180 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST

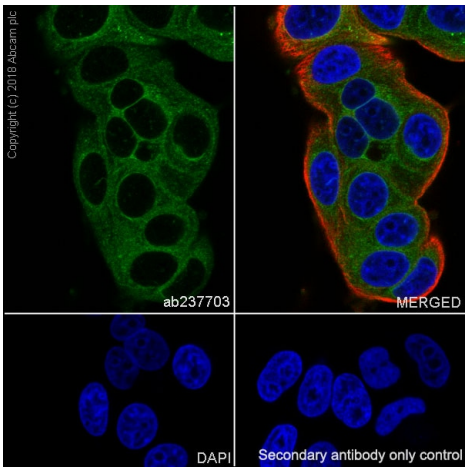


Immunoprecipitation - Anti-RAB10 antibody [MJF-R23] (ab237703)

RAB10 was immunoprecipitated from 0.35 mg A549 (human lung carcinoma cell line) whole cell lysate using ab237703 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab237703 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/5000 dilution was used for detection.

Lane 1: A549 whole cell lysate 10µg (input)
Lane 2: ab237703 IP in A549 whole cell lysate.
Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab237703 in A549 whole cell lysate.

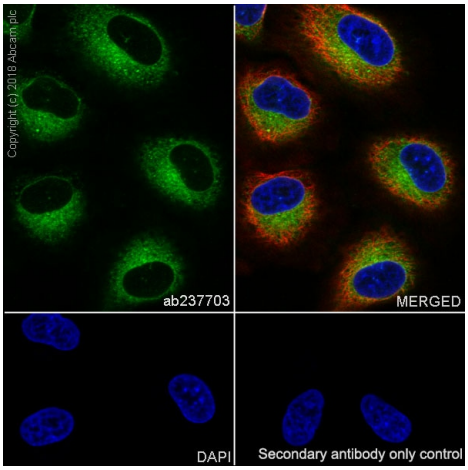
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-RAB10 antibody [MJF-R23] (ab237703)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (human breast adenocarcinoma cell line) cells labeling RAB10 (green) with ab237703 at 1/500 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution. Confocal image showing cytoplasmic staining in MCF7 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (red).

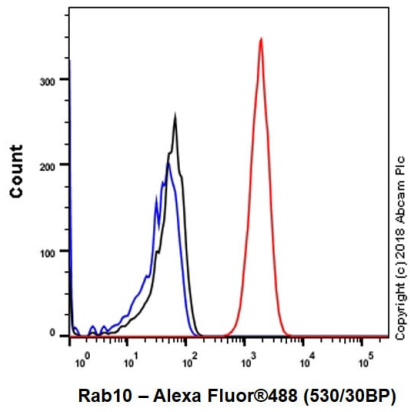
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-RAB10 antibody [MJF-R23] (ab237703)

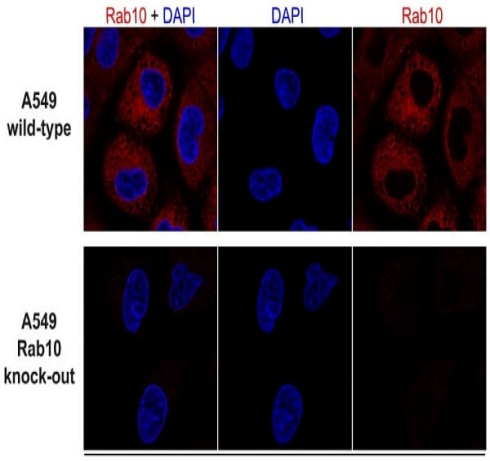
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma cell line) cells labeling RAB10 (green) with ab237703 at 1/500 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution. Confocal image showing cytoplasmic staining in A549 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-RAB10 antibody [MJF-R23] (ab237703)

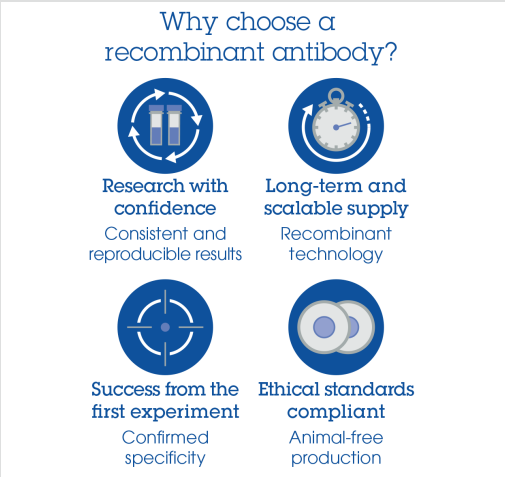
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized A549 (human lung carcinoma cell line) cells labeling RAB10 with ab237703 at 1/600 dilution (red) compared with the rabbit monoclonal IgG (**ab172730**) isotype control (black) and an unlabelled control (cells without incubation with primary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**)at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-RAB10 antibody [MJF-R23] (ab237703)

Image courtesy of Dr. Dario Alessi from University of Dundee

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% saponin permeabilized A549 wild-type and knock-out cells labeling RAB10 (red) with ab237703 at 0.5 µg/ml, followed by anti-Rabbit secondary at 1/1000 dilution. The nuclear counter stain is DAPI (blue).



Anti-RAB10 antibody [MJF-R23] (ab237703)

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